Test-specific Plan

The Efficacy test of EcoGuadian™ BWMS

(Shipboard scale)

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1. Test title

Efficacy test of EcoGuadian™ Ballast Water Management System (shipboard scale)

2. Test purpose

2.1 The objective of this test is to evaluate the biological efficacy of EcoGuadian™ Ballast Water Management System. (Based on the IMO shipboard test guideline)

2.2 We will determine elimination efficacy of organism lager than 50 μm, organism between 10 and 50 μm and bacteria by treatment of EcoGuadian™ BWMS.

3. Test site and schedule

3.1 The port of shipboard test

Test	Ballasting	Deballasting	Remark
1st test	Tolo (Hong Kong)	Donghae (Korea)	Invalid a)
2nd test	Kaohsiung (Taiwan)	-	Invalid a)
3rd test	Gamcheon (Korea)	Donghae (Korea)	Satisfied D-2
4th test	Gunsan (Korea)	-	Invalid a)
5th test	Tolo (Hong Kong)	Tolo (Hong Kong)	Satisfied D-2
6th test	Gunsan (Korea)	Gamcheon (Korea)	Satisfied D-2

a) Invalid, Test water condition as stipulated in D-2 not met

3.2 The test schedule

Test	Ballasting date	Deballasting date
1st test	2013.12.8~9	-
2nd test	2014.5.22	-
3rd test	2014.5.28	2014.5.30
4th test	2014.6.22	-
5th test	2014.7.21	2014.7.23
6th test	2014.9.16	2014.9.18

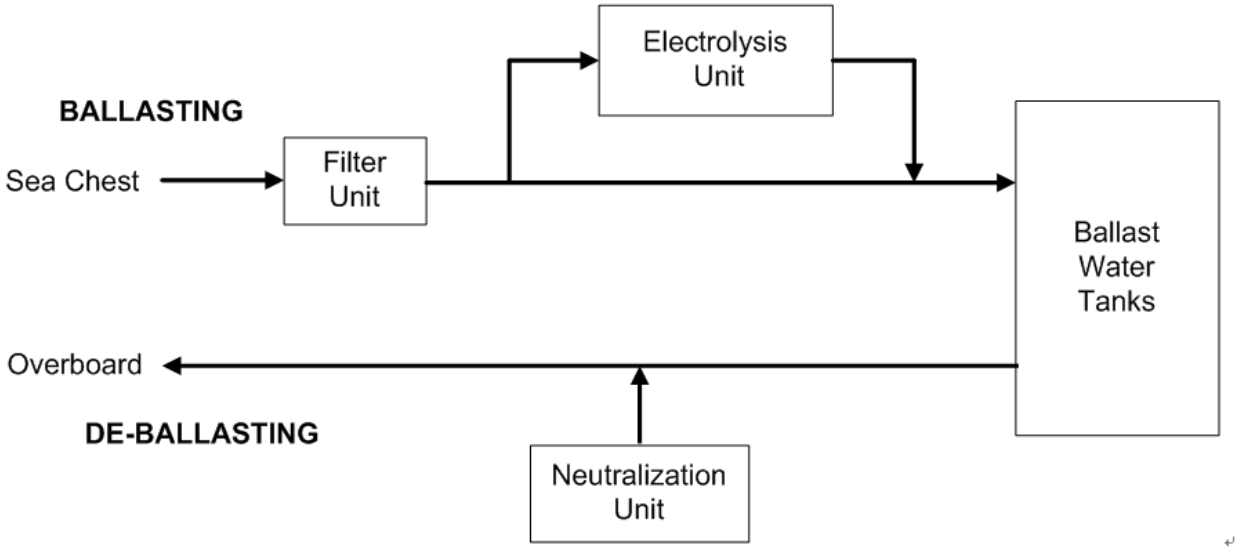
4. Sampling procedures

4.1 Test Ship introduction

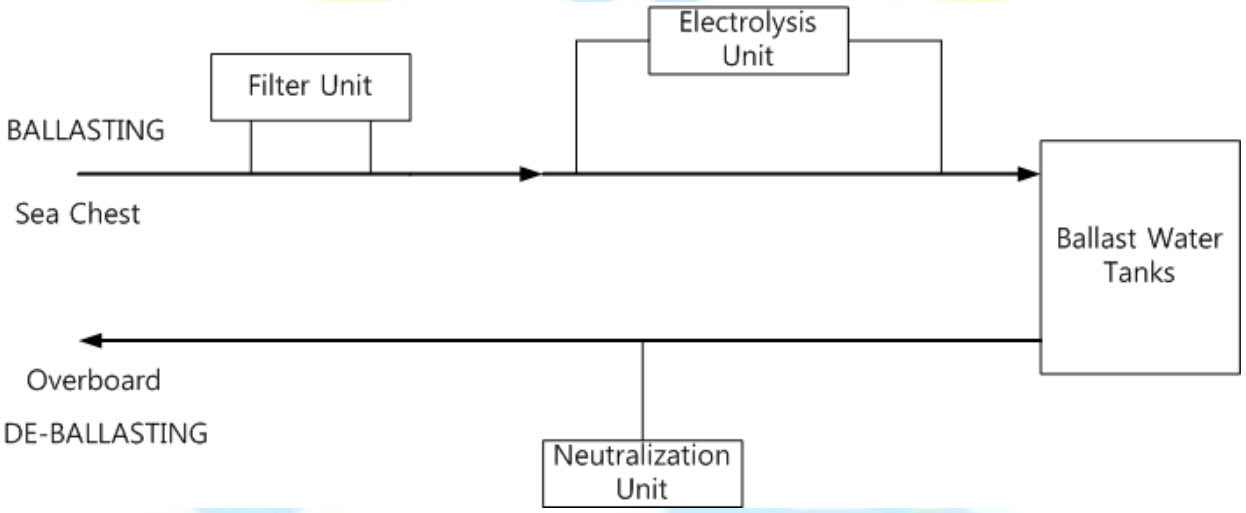
Ship Name	CHANG YAHNG
Ship Owner	SSANG YONG SHIPPING
Ship Builder	SHINA SHIPBUILDING
Hull No.	SAS380
IMO No	9121027
Ship Type	Bulk Carrier 10K
Ballast Pump Capacity	350 m^3 / h × 20 m
Class	KR

4.2 BWMS introduction

4.2.1 Treated mode

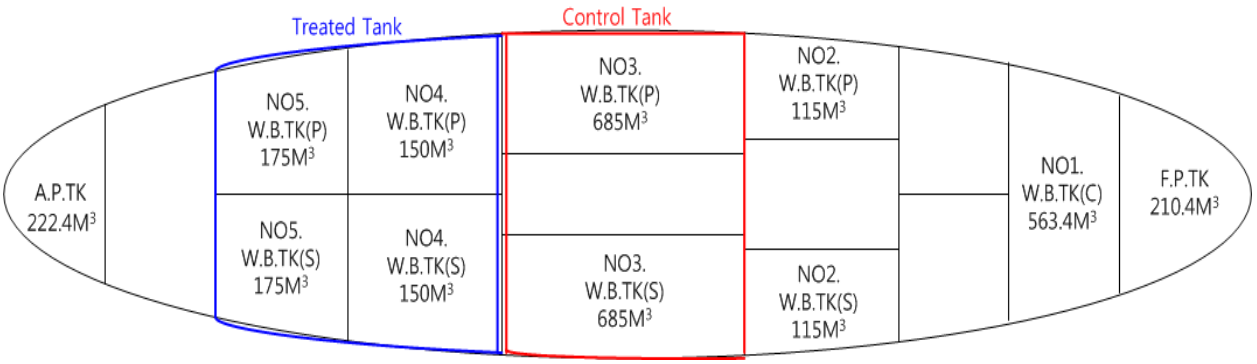


4.2.2 Control mode



Mode		Process
Treated	Ballasting	Sea Chest → Ballast pump → Filter(50㎛) → Electrolysis(side-stream) → Ballast Water Tank
	De-ballasting	Ballast Water Tank → Neutralization(sodium thiosulfate) → Ballast pump → Overboard
Control	Ballasting	Sea Chest → Ballast pump → Ballast Water Tank
	De-ballasting	Ballast Water Tank → Ballast pump → Overboard

4.3 Test Tank(Ballast Tank in SSANG YONG CHANG YAHNG)

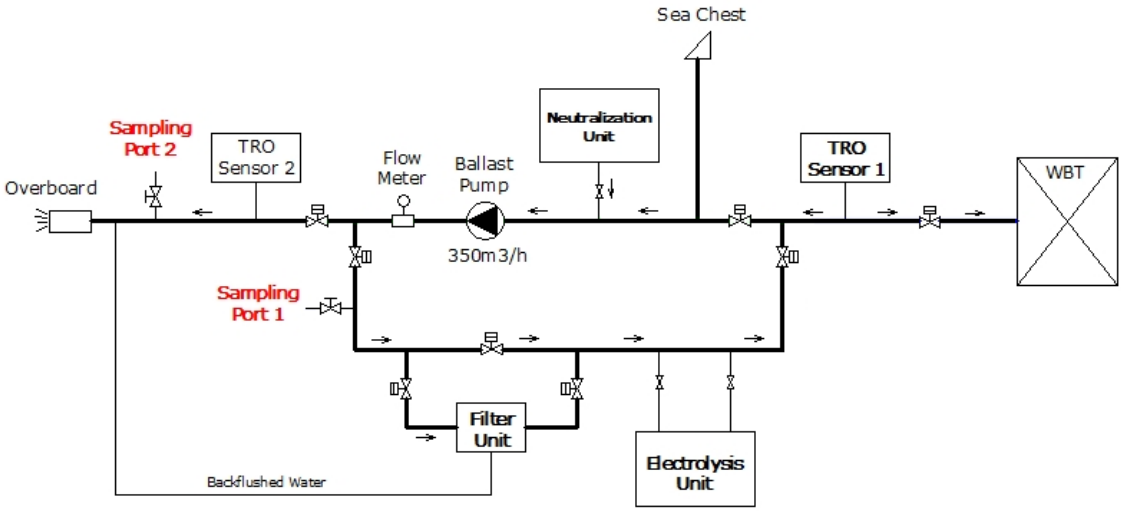


4.3.1 Tank capacity

Tank	Tank Number	Capacity
Treated Tank	No.4 WBT(S)	150 m^3
	No.4 WBT(P)	150 m^3
	No.5 WBT(S)	175 m^3
	No.5 WBT(P)	175 m^3
	Total	**650 m^3**
Control Tank	No.3 WBT(S)	685 m^3
	No.3 WBT(P)	685 m^3
	Total	**1370 m^3**

* Because of saftey, it selected the two tanks as a control tank and the four tanks as treated tank. It is to aim at ballasting or deballasting at the same time by using the port side and starboard.

4.4 Sampling point



Sampling port	Day	Sample name	Test Parameters
1	Day 0	Uptake water	Water parameters
			Organism
			Bacteria
2	Day *	Discharge Control / Discharge Treated water	Water parameters
			Organism
			Bacteria

4.5 Sampling regime

- Ballasting samples and deballasting samples (Beginning, Middle, End) are determined according to the uptake time and the discharged time.

Uptake water: the untreated water samples after ballasting at control tank, Day 0;

Discharged control water: the untreated discharged water samples in control tank, Day *;

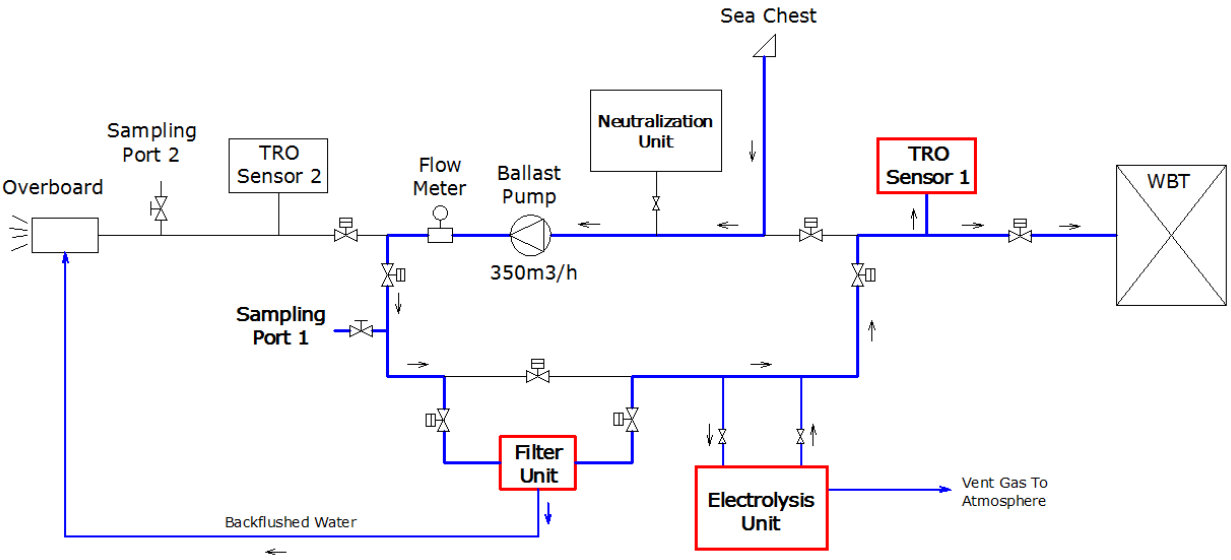
Discharged treated water: the ballasting water is treated and then neutralizes using the sodium thiosulfate, Day *;

4.5.1 Sample information

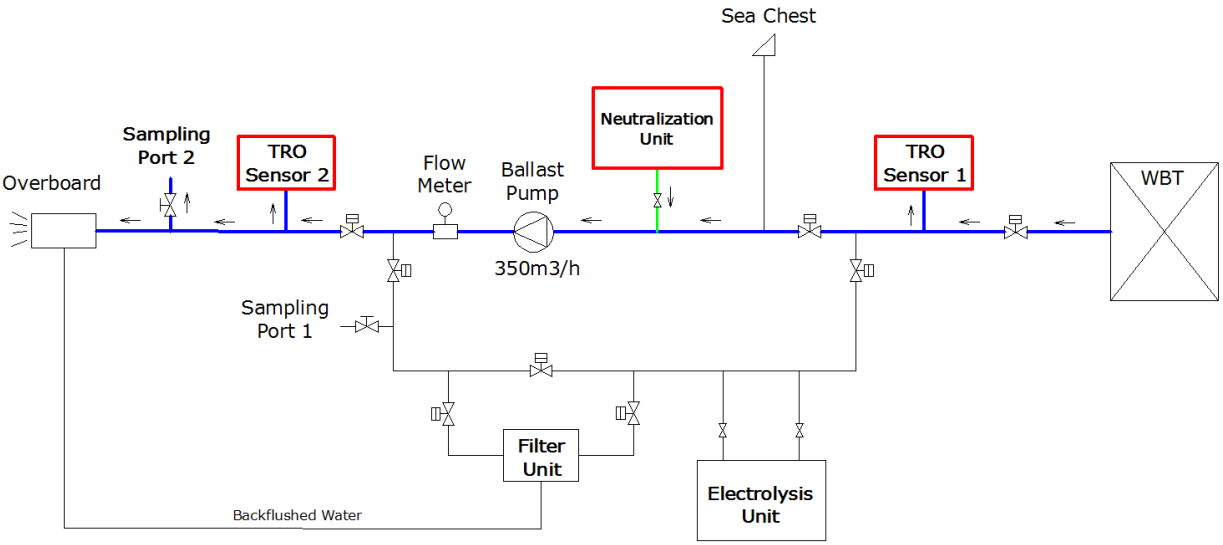
Day	Sample	Parameters	Number	Volume	Concentrate volume
Day 0	Uptake water	Water parameters	9	1~20 L	-
		DOC, POC, TSS	6	1~10 L	-
		≥ 50 µm sized organisms	3	1000 L	250 ~1000 mL
		10 ~ 50µm sized organisms	3	1~10 L	-
		Bacteria	3	1~2L	-
Day *	Discharge Control /	Water parameters	9	1~20 L	-
		≥ 50 µm sized organisms	3	1000 L	-
		10 ~ 50µm sized organisms	3	1~10 L	-
		Bacteria	3	1~2L	-
	Discharge Treated water	Water parameters	27	1~20 L	
		TRO	3	1~10 mL	-
		≥ 50 µm sized organisms	9	1000 L	250 ~1000 mL
		10 ~ 50µm sized organisms	9	1~10 L	250 ~1000 mL
		Bacteria	9	1~2L	-

*Remark - TRO is once measured in each discharge treated water (B, M, E)

4.5 Ballasting mode



4.6 Deballasting mode



4.7 Sampling procedures

- Collection and handling of field samples from the SSANG YONG "the CHANG YAHNG" will be undertaken by a team from BTP, using standard water sample collection methods and in accordance with the G8 Guidelines.
- Water samples will be taken from both the control (untreated) and treated tanks at two times intervals following treatment − at the ballasting (immediately after treatment, day 0), and at the discharge (deballasting or neutralizing agent treatment after * days, day *), and identified as numbered sampling points.
- All sampling equipments, apparatus and containers are prepared in accordance with EPA's Coastal 2000 Field Operation Manual. They are packed into exclusive cases.
- Water samples should be directly taken full up in a sample bottles after washing by sample water. When sample bottles are pre-preserved, the bottles should not be rinsed but be filled once with sample.
- Water parameters of samples are analyzed as soon as possible after collection at a field. The collected samples are transported to the laboratory in the CHANG YAHNG for the analysis.
- When the samples are arrived to laboratory, laboratory personnel receive the samples and entered the samples into the laboratory. The laboratory custodian will open the sample and carefully check the contents for evidence of leakage
- Sample handling will be performed so as to collect, store, submit to the laboratory and analyze representative samples using methods as specified in the test plans.

4.8 Test substances of Day 0

• Uptake water

Parameter	Sampling port	Sample ID
Organisms	1	HL-S01UW-B ~ HL-S06UW-B
Bacteria		HL-S01UW-M ~ HL-S06UW-M
Water parameters		HL-S01UW-E ~ HL-S06UW-E

4.9 Test substances of Day *

• Discharged control water

Parameter	Sampling port	Sample ID
Organisms	2	HL-S01CD*-B ~ HL-S06CD*-B
Bacteria		HL-S01CD*-M ~ HL-S06CD*-M
Water parameters		HL-S01CD*-E ~ HL-S06CD*-E

• Discharged treated water

Parameter	Sampling port	Sample ID
Organisms	2	HL-S01TD*-B-1 ~ HL-S06TD*-B-1
		HL-S01TD*-B-2 ~ HL-S06TD*-B-2
		HL-S01TD*-B-3 ~ HL-S06TD*-B-3
Bacteria		HL-S01TD*-M-1 ~ HL-S06TD*-M-1
		HL-S01TD*-M-2 ~ HL-S06TD*-M-2
		HL-S01TD*-M-3 ~ HL-S06TD*-M-3
Water parameters		HL-S01TD*-E-1 ~ HL-S06TD*-E-1
		HL-S01TD*-E-2 ~ HL-S06TD*-E-2
		HL-S01TD*-E-3 ~ HL-S06TD*-E-3

5. Test procedures

5.1 Test design

Test system	EcoGuadian^{TM} (shipboard scale)
Test substance	Uptake water : 100% (at ballasting) Discharge Control water : 100% (at deballasting) Discharge Treated water : 100% (at deballasting)
Dilution water	Distilled water(Autoclaved) or filtered natural seawater(Autoclaved)
TRO concentration	Treated : 9.0 mg/L Cl_2
Sampling time	Day 0, *

5.2 Test method

5.2.1 Water parameters measurement

1) Water parameters (temperature, pH, DO, salinity, turbidity) of samples at Field (SSANG YONG CHANG YAHNG) are measured using YSI according to BTP-BW-QI-01~04, 22.

2) Water parameters (TRO) of samples at Field (SSANG YONG CHANG YAHNG) are measured using Pocket colorimeter II according to BTP-BW-QI-06.

3) Water parameters (DOC, POC) of samples at BTP lab are measured using Vario TOC cube according to BTP-BW-QI-20~21.

4) Water parameter (TSS) of samples at BTP lab is measured according to SOP-BWMS-005.

5.2.2 Biological efficacy test

1) ≥ 50μm organism

① Concentration

• Sample can be concentrated with 32 μm net.

• Concentrated sample transfer into beaker and fill up to 250 ~ 2000 mL with filtered seawater.

② Analysis

• General method

- Analysis under stereo microscope with dark field (alive: movement /dead: lack of movement).
- 1 ~ 20 mL of concentration sample place on a counting chamber (sedgewick-Rafter cell or Bogorov counting chamber).
- The number of observations should be more than three.(control samples)
- Treated ballast water samples are observed all volume of concentrated sample.

2) 10 μm - 50 μm organism

① Concentration (Treated samples)

• Sample can be concentrated with 5 μm net. (If necessary, samples can be filtered by 150 μm plankton net.)

• Concentrated sample transfer into beaker and fill up to 250 ~ 2000 mL with filtered seawater.

② Analysis

• General method

- 0.1~1 mL of sample place on a counting chamber (sedgewick-Rafter cell).
- Waiting for 1~5 minutes for cell sinking.
- The number of observations should be more than three.

• Process of staining

- Making of FDA Stock Solution (Made in BTP lab)
- 1 mL of 100 % DMSO solution inject to a 5 mg of FDA, which keep frozen until use.
- Making of Working Solution (Made in BTP lab or field)

  Prepare by diluting the primary DMSO solution 100 times with stock solution.
- Each sample stained by adding 0.1 mL of the working solution to 3 mL sample.

  (Final concentration: 1.7 μL / mL FDA)
- Dyed sample place on a counting chamber.
- Waiting for 5~10 minutes for cell staining.
- Turn on mercury burner of microscope and apply to a fluorescent filter.
- Viable cells represent green color.
- Sample could be saved for up to 90 minutes without risking significant fluorescent degradation.

3) Heterotrophic bacteria

• SOP: SOP-BWTS-009

• Medium: 3M petrifilm - Aerobic Count Plate

• Method: Lift top film. Place 1 mL of sample onto center of bottom film. Release top film. Place spreader on top film over inoculum. Gently apply pressure on spreader.

• Sample volume: 1 mL

• Dilution water: Distilled water or filtered natural seawater (Autoclaved)

• Incubation: 35℃ ± 2℃, 24 ± 3 hours.

- Data analysis: Select the plate with the number of colonies in the acceptable range and count all colonies on selected plates promptly after incubation. And calculate count per 1 mL.

4) *Escherichia coli*

- Standard: EPA 1603 [*Escherichia coli (E. coli)* in Water by Membrane Filtration Using Modified membrane - Thermo tolerant *Escherichia coli* Agar (Modified mTEC) : 2006]
- SOP: SOP-BWTS-010
- Medium: Modified mTEC agar (DIFCO, Cat No 214880)
- Method: Filter sample using 0.45 µm membrane filter and then place filters on the top of plate.
- Sample volume: 10 mL ~ 1L
- Dilution water: Distilled water(Autoclaved) or filtered natural seawater(Autoclaved)
- Incubation: Incubate 35℃ ± 0.5℃ for 2 ± 0.5 hours. Transfer the plates to incubate 44.5℃ ± 0.2℃ for 22 ± 2 hours.
- Data analysis: Select the plate with magenta or red colonies, and calculate the number of *E. coli* per 100 mL
- Report results as *E. coli* CFU per 100 mL of sample.

5) *Enterococcus faecalis*

- Standard: EPA 1600 [*Enterococci* in Water by Membrane Filtration Using membrane-*Enterococcus* Indoxyl-β-D-Glucoside Agar (mEI): 2006]
- SOP : SOP-BWTS-011
- Medium: mEI agar (DIFCO, Cat No 214881)
- Method: Filter sample using 0.45 µm membrane filter and then place filters on the top of plate.
- Sample volume: 10 mL ~ 1L
- Dilution water: Distilled water or filtered natural seawater (Autoclaved)
- Incubation: 41℃ ± 0.5℃, 24 ± 2 hours
- Data analysis: Select the plate with colonies (regardless of colony color) with a blue halo. Calculate the number of enterococci per 100 mL
- Report results as enterococci per 100 mL of sample.

6) *Vibrio cholerae* O1, O139

- SOP: SOP-BWTS-012

• Medium: TCBS agar (DIFCO, Cat No 265020)

• Method

(1) Filter sample using 0.45 μm membrane filter and then place filters on the top of plate. Incubate the plates, protected from light at 35℃ ± 1℃ for 24 ± 2 hours.

(2) Each yellow sucrose fermenting colonies are placed on Non-salt Alkaline Pepton water. Incubate the plates at 36℃ ± 1 ℃ for 6-18 hours.

(3) Presumptive positive colonies are performed using slide agglutination assays by O1 and O139 antiserum for serological identification.

• Sample volume: 10 mL ~ 1L

• Dilution water: Distilled water (Autoclaved) or filtered natural seawater(Autoclaved)

• Data analysis: Select the colonies with agglutination O1 and O139 antiserum. Calculate the number of *Vibrio cholerae* per 100 mL

• Report results as *Vibrio cholerae* O1, O139 per 100 mL of sample.

5.2.3 List of equipment

Equipment	Management No.	Test range or Limits of detection	Model name	Manufacturer
Salimeter	A0015	0~10.00 mg/L	PR-100SA	ATAGO
Turbidimeter	A0008	0 ~ 4,000 NTU	2100AN	Hach
pH meter	A0127	0~14 pH range	Orion 3 star pH	Thermo
DO meter	A0010	1~100 mg/L	HQ40D	Hach
Vacuum pump	A0098	-	DOA-704-AC	GAST
Incubator	2030LD0001	-	VS1203S	Vision Science
Incubator	120201-03	-	JSGI-10T	JSR
Incubator	DLCDLE09464C	-	LIb-010-M	LabTech
Fluorescent microscope	A0031	X40,X100, X200,X400	BS51TR-32FB3F01 BX53	Olympus
Stereo Microscope	A0033/ A0145	X7~X115	SZX7-TR-SET SZX16-3136	Olympus
Auto clave	A108-2	-	VS-1321-100	Vision Scientific co.
Clean bench(Portable)	2060001381	-	700x400x500mm	
Ultrapure Water Purification System	A0045	-	Milli-Q Integal 10& 200L PEReseryer	Millipore
Electronic balance	A0055	-	EL204-IC	Mettler toledo
Pipette aid	A0094	0~100mL	4580800	Thermo
Deep freezer	A0070		CLN-70CW	Nihon

Multiple parameter	A0112	pH : 0 ~ 14 Units0.01 mg/L, 0.01 mS/cm , 0.01 ℃, 0.01 Unit, 0.01 m, 0.01 PPT, 0.1 NTU	YSI 6920 V2	YSI
Total Organic Carbon Analyzer	A0085		Vario TOC cube	Elementar
Electronic balance	A0123-1		EL204-IC	Mettler toledo
Electronic balance	A0125-1		MS3002S	Mettler toledo
Mass flask	B041, B042	1~100mL	100 mL	witeg
Mass cylinder	B062, B063	1~500mL	500 mL	witeg
Mass cylinder	B072, B073	1~100mL	100 mL	witeg
Mass cylinder	106426	250 mL		witeg
Mass cylinder	106426	250 mL		witeg
Thermometer and hygrometer	NLP012, NLP013, NLP016	-	PC-5000TRH-II	Testo
Micro pipette	A0093-8	0.5~10 µL	10123913	Biohit
Micro pipette	A0093-4	20~200 µL	9110842	Biohit
Micro pipette	A0093-3	0.1~1 mL	9110840	Biohit
Micro pipette	A0093-1	0.1~1mL	SL-1000XLS	Biohit
Micro pipette	A0093-1	0.1~1mL	SL-1000XLS	Biohit
Micro pipette	A0018-1	1~10mL	10mL	Eppendorf
Micro pipette	A0018-5	1~10mL	10mL	Eppendorf
Micro pipette	A0016-1	1~5mL	5mL	Eppendorf
Micro pipette	A0016-3	1~5mL	5mL	Eppendorf
Micro pipette	A0093-3	20-200 µL	200 µL	Biohit
Micro pipette	A0093-3	20-200 µL	200 µL	Biohit
Micro pipette	A0067-1001	1~1mL	1000 µL	Eppendorf
Micro pipette	A0016-4	1~5mL	5mL	Eppendorf
Micro pipette	A0093-2	20~200 µL	200 µL	Biohit
Micro pipette	A0093-5	1~20 µL	20 µL	Biohit
Micro pipette	A0093-7	1~10 µL	10 µL	Biohit
Micro pipette	A0067-1002	1~1mL	1000 µL	Eppendorf
Micro pipette	A0067-202	20-200 µL	200 µL	Eppendorf
Micro pipette	A0067-22	1~20 µL	20 µL	Eppendorf
Micro pipette	A0067-12	1~10 µL	10 µL	Eppendorf
Micro pipette	A0067-11	1~10 µL	10 µL	Eppendorf
Micro pipette	A0067-21	1~20 µL	20 µL	Eppendorf
Micro pipette	A0067-201	20~200 uL	200 µL	Eppendorf
Micro pipette	A0067-1003	0.1~1mL	1000 µL	Eppendorf

6. Validity

6.1 Water parameters

6.1.1 Measurement of water parameters should be performed at least three times.

6.1.2 Sample should be analyzed immediately at field and TSS, DOC, POC sample should be analyzed as soon as possible after arrival at BTP lab.(storage at below -20℃）

6.2 Biological efficacy test

6.2.1 Influent condition should be appropriate for the following IMO standards;

- The organism lager than 50 μm: ≥ 10^2 individuals/m^3
- The organism between 10 to 50 μm: ≥ 10^2 individuals/mL,

6.2.2 Average discharge results from the control water should be more than the values in regulation D2.1;

- The organism lager than 50 μm: ≥ 10 individuals/m^3
- The organism between 10 and 50 μm: ≥ 10 individuals/mL

6.3 Parameter precision, accuracy values and decimal units labeling for G8 testing SOP

Parameter	Resolution (Precision)	Accuracy	Decimal units labeling
Temperature	0.01 ℃	±0.15 ℃	The hundredths place
Salinity	0.01 ‰	±0.1 ‰	
Dissolved Oxygen	0.01 mg/L	±0.2 mg/L	
pH	0.01	±0.2	
Turbidity	0.1 NTU	±2 NTU	The tenths place
TRO	0.01 mg/L (0.02-2.00 mg/L)	CV < 20%	The hundredths place
	0.1 mg/L (0.1~8.0 mg/L)	CV < 20%	
TSS	0.1 mg/L	±5%, at 100 mg/L	The tenths place
DOC	80~120 %	CV < 20%	The thousandths place
POC	80~120 %	CV < 20%	

Phytoplankton	CV < 20%	-	Basically, a natural number (If the decimal point occurs, it would be specified the result rounded off to the first digit after the decimal point.)
Zooplankton	CV < 20%	-	
Heterotrophic Bacteria	CV < 20%	-	
Escherichia coli (E. coli)	CV < 20%	-	
Enterococcus group	CV < 20%	-	
Vibrio cholerae	CV < 20%	-	

7. Data and report

7.1 Data

7.1.1 Water parameters measurement

1) Temperature and salinity

Temperature and salinity were measured using an YSI 6920 multiparameter water quality sonde or HQ-40D. The sensing unit was calibrated once year by the manufacturer.

. 2) Dissolved oxygen (DO)

DO was measured using an YSI 6920 multiparameter water quality sonde or HQ-40D. The optical DO sensor was calibrated prior to the measurement according to the guidance provided by manufacturer. Maintenance should be carried out at least every year months.

3) pH

A pH meter measured using an YSI 6920 multiparameter water quality sonde or Orion 3 star pH meter. Measuring before correction should be performed daily.

4) Turbidity

A turbidity meter is measured using an YSI 6920 multiparameter water quality sonde or HACH 2100AN. The sensing unit and meter ware calibrated once a month**.**

5) TRO (Total Residual Oxidant)

Measurement of water parameter (TRO) is performed at least three times using measurement equipment (Model: Pocket colorimeter II)

6) DOC (Dissolved Organic Carbon), POC (Particulate Organic Carbon)

Measurement of water parameters (DOC, POC) are performed at least three times using measurement equipment (Model: vario TOC cube)

POC=TOC-DOC

7) TSS(Total Suspended Solids)

Measurement of water parameter(TSS) is performed at least three times.

Calculate non-filterable residue as follows:

$$\text{Non-filterable residue (mg/L)} = \frac{(A - B) \times 1000}{C}$$

where:

A = weight of filter (or filter and crucible) + residue in mg

B = weight of filter (or filter and crucible) in mg

C = mL of sample filtered

7.1.2 Biological efficacy test

Result data are presented mean value using calculation method as follows;

1) Survival rate

$$\text{Survival rate (\%)} = \frac{N_2}{N_1} \times 100$$

where:

N_1 = number of survival organism at the beginning

N_2 = number of survival organism at the end of the selected time interval

2) Heterotrophic bacteria

Select the plate with the number of colonies in the acceptable range and count all colonies on selected plates. And calculate count per 1 mL.

$$\text{CFU/ mL} = \frac{\text{The number of colonies}}{\text{Volume of sample (mL)}}$$

3) *Escherichia coli*

Select the plate with magenta or red colonies, and calculate the number of *E. coli* per 100 mL according to the following general formula:

$$\textit{E coli} \text{ / 100 mL} = \frac{\text{The number of } \textit{E. coli} \text{ colonies}}{\text{Volume of filtered sample (mL)}} \times 100$$

4) *Enterococcus faecalis*

Select the plate with colonies (regardless of colony color) with a blue halo. Calculate the number of enterococci per 100 mL according to the following general formula:

$$\text{Enterococci / 100 mL} = \frac{\text{The number of enterococci colonies}}{\text{Volume of filtered sample (mL)}} \times 100$$

5) *Vibrio cholera* O1, O139

Count positive results with slide agglutination assays by O1 and O139 antiserum for serological identification. Calculate the number of *Vibrio cholera* O1, O139 per 100 mL according to the following general formula:

$$\textit{Vibrio cholera}\text{ O1, O139 / 100 mL} = \frac{\text{The number of }\textit{Vibrio cholera}\text{ O1, O139}}{\text{Volume of filtered sample (mL)}} \times 100$$

7.1.3 Coefficient of variation (CV)

Coefficient of variation for each replicate should be calculated as follows.

$$\text{CV (\%)} = \frac{Y}{X} \times 100$$

where:

X: The mean value for respective replicate

Y: Standard deviation for respective replicate

7.2 Report

- Result
- Appendix